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EDITORIALS

We, your Editoral Staff, having made an earnest effort to produce a publication of official origin for the members of the American Society of Clinical Laboratory Technicians, present herewith our first issue.

We hope that posterity will favor this, our beginning; that time will develop a publication which is worthy of the name it bears, and that it shall be classed as an outstanding endeavor in the field of laboratory procedure.

We earnestly solicit the support of each member of this organization, and ask that you utilize this medium, which has long been the cherished desire of the ethical laboratory technician.

Our ideals have been guided by the adopted "Laboratory Technicians' Creed" printed in this, our initial issue. May the undertaking built upon the foundation of such ideals be a guiding factor to our chosen profession.

Chairman of Publication Committee

THE CLINICAL PATHOLOGIST AND THE LABORATORY TECHNICIAN

Time was, and that not so long ago, when the physician specializing in clinical pathology carried out in person all the technical procedures including the humble task of cleaning the glassware. Today in our highly organized society with its striving for efficiency

such a state of affairs is rightly considered as an economic waste. The technician can safely be entrusted to perform many laboratory tests with perhaps better skill than the director while the latter can utilize his talents not only in controlling and coordinating the work of his assistants but in interpreting and evaluating the findings while acting as a consultant to the physician in attendance on the case.

It is told of Ribbert, the great German pathologist, that he not only cut his own tissue sections and stained them, but that he also made with his own hand the drawings for his well known textbooks. Much as we may admire his versatility, such profligacy of time and patience could not but have robbed medicine of further profitable fruit of his research.

This is an era of division of labor, also of mass production when we take into consideration the thousands of hospitals now requiring routine urine and blood examinations on all patients, not to mention the numerous clinics, dispensaries, and private practitioners demanding laboratory work, it is inconceivable how this function could be performed without the aid of a multitude of trained laboratory technicians.

These indispensable assistants, however, are not mere automatons performing their duties like robots. They are far from being factory operatives performing repetitions and monotonous tasks. The clinical pathologist expects the technician to know the why and wherefore of the separate steps in a given procedure, to exercise initiative and ingenuity and continually to improve the mind and the technique.

The clinical pathologist hails with gratification the formation of a national organization of technicians such as the A. S. C. L. T. and particularly rejoices in their successful ambition to publish their own journal. The launching of this periodical betokens an abundance of creative energy which promises much for the future status of this useful vocation both from a scientific as well as a sociological standpoint.

The clinical pathologists wish the members of the A. S. C. L. T. continued good luck in this new venture.

PHILIP HILLKOWITZ

THE LABORATORY TECHNICIAN

Past, Present, and Future

Until the advent of bacteriology and the development of the laboratory era in medicine, the clinical laboratory technician was unrecognized. However, during the last half century a great many laboratory methods have been devised for the purpose of assisting the physician in proper diagnosis, treatment, and prognosis. Soon

the physician found himself physically unable to perform the many necessary laboratory tests and thereupon the "dieners" and "bottle washers" metamorphosed into laboratory assistants and technicians. Newer methods of technique in all of the various divisions of the clinical laboratory followed one after another in rapid succession. Many advancements were made in clinical bacteriology, serology, and analytical methods. Blood chemistry became a routine procedure and hematology took the place of the ordinary "blood count."

During this rapidly changing period, those engaged as technicians picked up their training in various ways. Some had the advantage of college training; others were not high school graduates. As the demand for technicians increased, many recruits were pressed into service, comparatively few of whom were well trained.

Finally, notice was taken of this chaotic condition in laboratory medicine as illustrated by the following references:

1. Gradwohl, R. B. H.: The training and proper recognition of the laboratory technician. Jour. Lab. and Clin. Med., August, 1921, VI., 644-647.
2. Grant & Wilson: A plea for the standardization of the training for laboratory technicians. Jour. Lab. and Clin. Med., June, 1922, VII., 562-563.
3. Kolmer, J. A.: State licensure applied to laboratorians. Jour. Am. Med. Assn., September 9, 1922, LXXIX., 861.
4. Moore, J. J.: Standardization of clinical laboratories. Jour. Am. Med. Assn., September 9, 1922, LXXIX., 863.
5. King, W. E.: The training of the laboratory technician. Minnesota Medicine, April, 1923, 233-238.
6. Ikeda, K.: Standardization of clinical laboratories and technicians. Minnesota Medicine, 6:397-400, June, 1923.

From one of the above mentioned papers (King) which was read before the meeting of the Minnesota State Medical Association, Minneapolis, October, 1922, the following paragraph is quoted:

"Clinical laboratory technicians should receive proper recognition in the form of an official certificate or license from state boards of health or other authorities. Moreover, technicians should be graded according to ability, training, and experience. Licenses should be issued to those who merit recognition as chief technicians or first assistants to the directors of laboratories, and to assistant technicians. A certain period of successful work as assistant technician, regardless of previous educational advantages, should entitle one to apply for license as chief technician."

Little need be said concerning the present status of the laboratory technician. The mighty change which has taken place is witnessed by the appearance of this first issue of the Official Publication of the American Society of Clinical Laboratory Technicians and by the information contained in its pages. It is now well recognized that the specialized training of the technician is of as much importance

to the physician as is the professional training of the pharmacist who fills his prescriptions and that of the nurse who carries out his orders in the sick room and in the hospital ward.

Proper recognition of the important work of the laboratory technician, the standardization of courses of training for student technicians, and official licensure, definitely point to the position which this work is to occupy in medicine. Destiny has decreed that clinical laboratory technique shall become recognized as one of the true professional vocations.

W. E. K.

MICROSCOPIC SLIDE PRECIPITATION TESTS FOR THE DIAGNOSIS AND EXCLUSION OF SYPHILIS

By B. S. KLINE, M.D.,

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The microscopic slide precipitation tests for the diagnosis and exclusion of syphilis (1) in addition to utilizing certain desirable test conditions such as concentrated emulsion, optimal proportions with serum, acceleration by agitation, etc., as do flocculation tests in general use, are characterized as follows:

In the first place, the antigen employed is the acetone insoluble fraction (phosphatids) only of alcoholic heart extract. [Of all the substances in alcoholic tissue extract, this particular small fraction had previously been found to be the most potent and most specific Wassermann test antigen by Noguchi and Bronfenbrenner (2), Browning, Cruickshank and M'Kenzie (3) and Neymann and Gager (4)].

Secondly, the antigen emulsions employed in the microscopic slide precipitation tests for syphilis are more stable and more uniformly sensitive than those in general use. Antigen emulsions for most precipitation tests are relatively unstable, composed of aggregates which vary in size and shape as quantitative relationship, speed of mixture and temperature of cholesterinized antigen and salt solution are varied and accordingly give unavoidably variable results. Antigen emulsions prepared as for the slide tests with the cholesterin precipitated first and with the crystals then coated by antigen are relatively stable, composed of aggregates differing but little in size and shape and give uniformly sensitive results. Furthermore the plate like units of the microscopic slide precipitation test emulsions with flat surfaces are agglutinable by a minimal amount of reagin.

Lastly, the reactions are carried out on optimal open polished surfaces of microscopic slides in chambers of optimal proportions and the results are read accurately with ease through the microscope at a magnification of about 120 times.

In practice the microscopic slide precipitation tests for syphilis have been found to be comparatively simple, specific and sensitive.

Examined through the microscope (high magnification) the clumps of positive microscopic slide precipitation tests may be seen to be composed of the coated plates of the emulsion.

Although not microscopically visible, there is evidence as presented by Eagle (5) that the mechanism of clumping of the antigen particles by syphilitic serum, is similar to that of specific bacterial agglutination, and due to a combination of altered globulin and antigen particle resulting in a surface with hydrophobic character. Not only does the reagin in syphilis clump proper antigen emulsions much as antipneumococcus serum (type 1) clumps (type 1) pneumococci but also like antibody in pneumococcus infection, it is apparently produced only as long as the infection is active and then, soon thereafter, is no longer demonstrable.

The suggestion that this reagin may be an autoantibody to the products of tissue destruction in syphilis was first advanced by E. Weil (6). This view has been supported by the work of Landsteiner (7) and Sachs, Klopstock, and Weil (8).

Materials for the Slide Tests

Sera:

These are prepared as for the Wassermann test, care being exercised that they contain no red blood cells, or foreign particles. (They are heated at 56° C. for thirty minutes.)

When blood is obtainable in small quantity only it is advisable to collect this in a narrow test tube (about 8 to 9 mm.) and to handle it in the same manner as a larger sample from the vein.

When blood is obtainable in very small quantity only it is advisable to collect this in a narrow glass tube with a capillary end. The end is then sealed, a narrow rod is passed through the open end to free the clot from the wall and after the tube is centrifuged at high speed, it is placed in a water bath at 56° C. with water above the upper level of the serum. After inactivation, the tube is filed and broken just above the clot and the serum allowed to run into or is drawn into a 1 cc. pipet, graduated in hundredths.

Glassware:

Microscopic slides 2 x 3 inches as purchased are rubbed on both sides with Bon Ami paste (prepared by breaking up a cake of Bon Ami in a small quantity of hot water). As soon as the paste is dry (in about five minutes) it is completely removed from the slide with a soft muslin cloth. For convenience the slides covered with paste may be stuck to each other, allowed to dry, and cleaned at any time.

Upon clean slides for the heated serum tests, 12 paraffin rings, each with an inside diameter of 14 mm. are mounted. (Inasmuch as the slide test results are influenced by the surface area of the chambers it is important that the paraffin rings be thin ones. With

a little practice the required amount of paraffin can be ascertained. At first, it may be difficult to make complete rings. These incomplete rings may be completed by applying the loop a second time to the open areas.)

For the spinal fluid tests double ring slides are prepared as follows: Upon the clean slide a steel mold 3 2/16 x 2 3/16 x 1/8 inches with two central wells 1 9/16 inches in diameter is placed. A metal disc 1 5/16 inches in diameter and 3/16 of an inch thick is then placed in the center of each well. The space between them is filled with hot wax (2 parts ordinary vaseline and 1 part parowax) from a 10 cc. glass syringe. After the mixture cools a few minutes, each disc is elevated from the slide and separated from the wax wall by turning the central screw handle a few times to the right (holding mold down at edge). After the disc is freed, it is lifted out. The mold is removed by inserting a thin blade between it and the slide.

Pipets:

The pipets needed for delivering sera, and spinal fluid, and those for preparing the antigen emulsions are the ordinary finely graduated 0.2 to 10 cc. pipets. The pipet for the 1% acetic acid solution is a 0.2 cc. pipet graduated in 0.001 cc. The pipets for delivering the antigen emulsions are Wright pipets made from glass tubing 6 to 10 mm. in diameter with the tubes about 0.5 mm. in outside diameter, delivering a drop equal to about 0.008 cc. (62 drops per 0.5 cc.).

Instrument for Making Paraffin Rings:

This is essentially the instrument proposed by Green (9). A piece of soft iron wire (No. 28) is wound twice tightly about a test tube (about 15 mm. in outside diameter) forming a double loop and leaving a double shaft about an inch in length. The two shafts are then twisted together to within a quarter of an inch of the free end. After removing the looped wire from the test tube, a piece of linen thread (No. 12) is started from the free end of the shaft after being fastened here by a single twist of the free ends. Three long turns are made reaching the loop which is then tightly wound with the thread. The winding is continued up the shaft to the free end where it is fastened between the two ends of the wire by twisting them. The loop is then bent at right angles to the shaft. It is then reshaped by working the loop against the bottom of the test tube mentioned above. The shaft is then inserted into the handle of a teasing needle or into a straight hemostatic forceps.

The paraffin rings are made by dipping the instrument into smoking paraffin (about 120° C.) draining quickly at one point and transferring the remainder to the glass slide.

Slide Holders (For 3 x 2 inch slides):

The slide holder is a wooden lid of a slide box (3½ x 6¾ x ½ inches) containing an easily fitting thin wooden shelf having a small handle at each end.

Salt Solutions:

0.85% Sodium chloride (C.P. or reagent, Merck) solution used in the tests is prepared with distilled water having a pH of about 6. (Such water gives a lilac color when one drop of chlorphenol red indicator (LaMotte) is added to 0.25 cc. of it in a small chamber). Distilled water having a pH of 5.2 or less gives a yellow color with this indicator and is not satisfactory.

1% *Acetic Acid:*

(C. P. Reagent.) It is advisable to use no less than 1 cc. of acid (delivered from a 1 or 2 cc. pipet) and accordingly 99 cc. of distilled water.

Antigen: *

The purified antigen used in the microscopic slide precipitation tests for syphilis is prepared as follows:

Two-hundred grams of dried heart powder (Difco) is placed in a 2 liter Erlenmeyer flask.

One liter of absolute ethyl alcohol (99+ per cent) (Rossville Commercial Alcohol Corp., Lawrenceville, Ind.) is added.

After the flask is stoppered with a cork covered with tin foil, it is shaken vigorously by hand at intervals for two hours. Better still two wide mouth bottles (Difco bottles for one pound beef heart powder) each with 100 grams of beef heart powder and 500 cc. of absolute ethyl alcohol (99+ per cent) are shaken vigorously in a machine for two hours. (This short extraction removes almost all of the desired antigenic substance in the powder.)

The extract is filtered into a liter cylinder through good grade filter paper of medium texture (Schleicher and Schull No. 597, 38.5 cm.)

During filtration the mixture is stirred with a wooden tongue depressor and toward the end pressed with the cork until the powder is quite dry.

The extract (about 775 cc.) is placed in the refrigerator at 8° to 10° C. for twenty-four hours.

During this time a fairly heavy white precipitate settles out. This is filtered off and the filtrate in a large evaporating dish is concentrated on a water-bath at 45° to 50° C. determined by a thermometer bulb within the extract. During evaporation of the alcoholic extract an irregular festoon appears at the periphery. When the extract reaches the proper concentration the festoon disappears and the margin of the concentrated extract is sharp.

* Standard materials including antigen for the microscopic slide precipitation tests for syphilis may be obtained from the LaMotte Chemical Products Company, McCormick Building, Baltimore, Md.

The extract is now poured quickly into 500 c.c. of acetone, C.P. (Coleman and Bell) at 50° C. in a large evaporating dish.

The dish is then placed in an air incubator at 37° C. for fifteen minutes after which the acetone is decanted leaving a soft yellow brown wax adherent to the side of the dish. (Longer periods of precipitation and precipitation at lower temperatures permit of precipitation of adventitious substances as well and such antigens give more sensitive and less specific results).

The dish is then placed on a water bath or in an air incubator at 50° C. until the little acetone remaining has evaporated (about thirty minutes).

The wax is then worked together and placed in a glass stoppered bottle. Then 80 c.c. of absolute ethyl alcohol (99+ per cent) that has been kept in an air incubator at 50° to 56° C. for one-half hour or longer, is added and after a few minutes shaking the bottle is placed in an air incubator at 50° C. and shaken gently after fifteen minutes and again after thirty minutes, when it is removed from the incubator and placed in the refrigerator at 8° to 10° C. for forty-five minutes.

The solution is then filtered and the filtrate is evaporated down at 45° to 50° C. resulting in a soft brown wax (antigen wax). The wax is weighed and to each gram in a glass stoppered bottle, 10 c.c. of absolute ethyl alcohol (99+ per cent) (at 50° to 56° C.) is added. After the bottle is shaken for a few minutes it is placed in an air incubator at 50° C. for thirty minutes, and then shaken a few minutes.

The slightly turbid solution is then placed at 8° to 10° C. for about an hour and then filtered. The resultant clear filtrate is the antigen, and contains about 8.75 per cent of the alcohol-treated acetone-insoluble wax.

The average yield of antigen wax from ½ pound of beef heart powder is 3.5 to 4 grams. The discarded acetone-soluble lipoidal residue (impurities) ordinarily weighs at least three times this amount.

The antigen keeps best at room temperature hermetically sealed in glass containers. In small necked glass stoppered bottles (opened from time to time) kept at room temperature it shows no appreciable change in specificity or sensitivity for at least six months. (As stated above it is important to follow the steps outlined in minute detail. Too long a precipitation in acetone, precipitation at too low a temperature and use of improper chemicals for instance result in waxes which contain some impurities that determine a sensitivity greater than standard).

Preparation of Antigen Emulsions for Diagnostic and Exclusion Slide Tests of Heated Serum

Formula:

0.85 cc. of distilled water (pH about 6).
1.0 cc. 1% cholesterin (C.P. Pfanstiehl) in absolute ethyl alcohol (99+ per cent).†
0.1 cc. antigen.
2.45 cc. 0.85% sodium chloride (C.P. or reagent, Merck) solution (pH about 6).

The technic of preparing the emulsion according to the above formula is as follows: Into a 1-ounce bottle the required amount of distilled water (pH about 6) is pipetted.

The bottle is held at an angle, and the 1% cholesterin in absolute ethyl alcohol (99+ per cent) is allowed to run along the side of the neck of the bottle.

The bottle is gently rotated from the neck for twenty seconds. It is held at an angle again, and the proper amount of antigen is pipetted against the side of the neck of the bottle from a finely graduated pipette.

The bottle is promptly stoppered with a cork and shaken vigorously (the fluid thrown from bottle to cork and back) for one minute.

Lastly, the 0.85% sodium chloride solution is allowed to run in quite rapidly, the bottle is stoppered again and shaken less vigorously than previously for one minute.

The emulsion, when examined under the microscope at a magnification of about 120 times, shows numerous very fine particles, but no clumps whatever.

For Diagnostic Test

Place 1 cc. or more of the emulsion in a narrow test tube (12 mm. inside diameter) in a water-bath at 35° C. (beaker of water in usual laboratory air incubator at about 37° C.) for fifteen minutes. The emulsion as soon as heated is ready for use.

For Exclusion Test

Place 2 cc. of the emulsion in a narrow test tube (12 mm. inside diameter) in a water-bath at 56° C. for fifteen minutes. Then pour into a 3 x 1 inch tube and centrifuge for fifteen minutes (eighth setting Rheostat, Centrifuge Size 1, Type SB). Decant the fluid and, with the

† The 1% cholesterin solution for the emulsions is prepared in about forty-five minutes by placing the cholesterin flakes and absolute alcohol in a glass stoppered bottle in an oven at 50° to 56°C. and shaking gently a few minutes at fifteen minute intervals. The solution kept in the incubator at 37°C. is thoroughly satisfactory for use as long as two months.

The sensitivity of an emulsion is greatly influenced by the quantity of cholesterin present. The sensitivity is likewise influenced by the quality of the cholesterin used. The cholesterin (Pfanstiehl, C.P.) that has been found uniformly satisfactory in the slide tests is flaky, pearly and readily soluble to 1% in absolute ethyl alcohol (99+ per cent). Powdery, white cholesterin incompletely soluble to 1% in absolute alcohol (99+ per cent) has been found to give too sensitive results.

tube inverted, dry the inside of the tube with a cloth almost to the level of the sediment. To the sediment add 1.5 cc. of 0.85% sodium chloride solution. Transfer to a narrow tube for use.

These emulsions, kept at room temperature, are satisfactory for use for forty-eight hours after preparation.

Diagnostic and Exclusion Microscopic Slide Precipitation Tests for Syphilis with Heated Serum.

1. Place three heated serum test slides each with twelve small chambers, on a tray in a small holder.

2. Into each of the thirty-six rings, pipet, 0.05 cc. of the heated serum to be tested (eighteen sera in duplicate).

3. After all the sera are pipetted, 1 drop of the diagnostic test antigen emulsion (about 0.008 cc.) is allowed to fall from a Wright pipet into one of the two portions of each serum. Into each of the other eighteen duplicate sera a similar drop of exclusion test antigen emulsion is allowed to fall from a Wright pipet.

4. The slides in the holder are rotated on a flat surface for four minutes.

5. The results are examined at once through the microscope at a magnification of about 120 times (low power 16 mm. objective, eyepiece 12) with the light cut down as for the study of urinary sediments and reported in terms of pluses according to the degree of clumping and the size of the clumps.

Any spilling from the chamber makes the reaction therein unsatisfactory, and the serum concerned should be re-tested.

If sufficient serum is available the exclusion test for syphilis may be done with 0.3 cc. heated serum in a chamber similar to that employed for the spinal fluid test (33 mm. in diameter) and one drop (about 0.008 cc.) of emulsion made by suspending the sediment of 8 cc. of exclusion test emulsion (centrifuged 15 minutes at 8th rheostat setting) in 1 cc. of 0.85% salt solution.

Preparation of Antigen Emulsions for Diagnostic and Exclusion Slide Tests of Spinal Fluid.

Formula:

0.85 cc. distilled water (pH about 6).
1.25 cc. of 1% cholesterin (Pfanstiehl C.P.) in absolute ethyl alcohol (99+ per cent).
0.1 cc. antigen.
2.2 cc. of 0.85% sodium chloride (C.P. or reagent, Merck) solution (pH about 6).
8.8 cc. of the emulsion are made by using double the quantities given in the formula.

For Diagnostic Test

Place 4 cc. of the emulsion in a narrow test tube (12 mm. inside diameter) in a water-bath at 35° C. for fifteen minutes. Then pour into a 3x1 inch tube. Centrifuge for fifteen minutes (eighth setting Rheostat, 1, S.B.) Decant the fluid and, with the tube inverted, dry the inside of the tube with a cloth almost to the level of the sediment. To the sediment add 1 cc. of 0.85% sodium chloride solution (pH about 6).

Transfer to a narrow test tube for use.

For Exclusion Test

Place 4 cc. of the emulsion in a narrow test tube (12 mm. inside diameter) in a water-bath at 50° C. for fifteen minutes. Then pour into a 3x1 inch tube. Centrifuge for fifteen minutes (eighth setting Rheostat, 1, S.B.) Decant the fluid and, with the tube inverted, dry the inside of the tube with a cloth almost to the level of the sediment. To the sediment add 1 cc. of 0.85% sodium chloride solution (pH about 6).

Transfer to a narrow test tube for use.

These emulsions, kept at room temperature, are satisfactory for use for twenty-four hours.

Diagnostic and Exclusion Microscopic Slide Precipitation Tests for Syphilis with Spinal Fluid.

Preliminary procedures with spinal fluids.

Spinal fluids, turbid with exudate, blood or bacteria, or containing injected substances including horse serum, are unsatisfactory for testing. Spinal fluids with slight turbidity or few particles are centrifuged at high speed for ten minutes, and the clear fluid is withdrawn or decanted.

Place the required number of test tubes, 6 x 5/8 inches, each containing 5 cc. of Benedict's solution (1909-1910), in a beaker (Pyrex). Add water halfway to the top. Heat. Keep the tubes in vigorously boiling water for five minutes.

Place the tubes in a rack. After making certain that no copper reduction has occurred in any of the tubes, add to each tube, properly numbered, 0.5 cc. of spinal fluid. Shake each tube vigorously for ten seconds.

Replace the tubes in the beaker. Add water halfway to the top. Heat. Keep the tubes in vigorously boiling water for five minutes.

Replace the tubes in the rack, inspecting each immediately after removal from the beaker, for precipitate indicating presence of sugar.

Spinal fluids giving a negative reaction for sugar in the above test are unsatisfactory for testing for syphilis. These are fluids that have been acted upon by bacteria either inside or outside of the body. In the former case (bacterial meningitis) in which organisms and ferments of the exudate have acted upon the sugar, the fluids

may contain substances that give positive or unsatisfactory reactions in various tests for syphilis. Bacterial contamination subsequent to withdrawal of spinal fluids from cases of syphilis causes a steady loss both of sugar and of the specific reacting substance, if the fluids are kept at room temperature.

On the other hand, if spinal fluids containing sugar when withdrawn from the body are kept at low temperature (8° to 10° C.), they continue to give a positive reaction for sugar with the test described above for several weeks, and syphilitic spinal fluids under these conditions show no appreciable loss of specific reacting substance for at least a week.

The clear and cleared spinal fluids which give a positive reaction in the sugar test described above are then tested as follows:

Diagnostic and Exclusion Microscopic Slide Precipitation Tests for Syphilis with Spinal Fluid.

Into each of twelve chambers (33 mm. in diameter) on six glass slides (in a holder tilted slightly by placing a small metal bar ⅛ inch thick under one long side) deliver 0.05 cc. of 1% glacial acetic acid solution from a 0.2 cc. pipet graduated in thousandths. (It is of the utmost importance that the 1% acetic acid be carefully prepared since this reagent in stronger concentration will precipitate the emulsion).

Into each chamber allow 0.25 cc. of the spinal fluid to be tested (six spinal fluids in duplicate) to fall from a 1 cc. pipet graduated in hundredths. Hold the pipet directly above the acid and lastly touch the tip of the pipet at some dry portion of the chamber.

Rotate the slides in the holder on a flat surface with moderate vigor for one minute.

Into one-half of the chambers allow one drop (about 0.008 cc.) of diagnostic antigen emulsion to fall from a Wright pipet.

Into each of the duplicate spinal fluids one drop (about 0.008 cc.) of exclusion antigen emulsion is allowed to fall from a Wright pipet.

Rotate the slides in the holder on a flat surface with moderate vigor for one minute to distribute the antigen and then for four minutes move the holder gently but rapidly (about three complete movements a second) back and forth a distance of ¼ to ½ inch.

The results are examined at once through the microscope at a magnification of about 120 times (objective 16 mm. eyepiece 12) with the light cut down as for the study of urinary sediments and recorded in terms of pluses according to the degree of clumping and the size of the clumps. (For ease in reading the results, the slide is tilted on a piece of metal ⅛ inch thick, ½ inch wide, and 4 inches long, placed on the stage).

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THE VALUE OF THE SCHILLING HEMOGRAM IN CLINICAL HEMATOLOGY*

A Classification of the Leukocytes

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Hewson discovered the leukocytes, Virchow recognized their importance in pathologic processes and Ehrlich introduced a staining method that made it possible to study the morphology of these elements.

Ehrlich divided the leukocytes up into two systems, the bone marrow and the lymphoid tissue, into granulocytic and non-granulocytic respectively. Ehrlich further classified the granulocytes into three groups, the neutrophils, eosinophils and basophils, building these nomenclature upon the staining characteristics of the cytoplasmic granules.

A third system was added by Schilling, the reticuloendothelial tissue which gives rise to another group of granulocytes, the monocyte. Although much is known about the leukocytes, there is still much controversy as to their origin. Three schools today are engaged to settle the question once and forever, the monistic (uni-

* *Read before the Meeting of the American Society of Clinical Laboratory Technicians, Cleveland, Ohio, June* 11-13, 1934.

tarian), dualistic and the trialistic. The latter having as its leader, Prof. Schilling, contending that each group of the leukocytes has an individual stem cell (undifferentiated leukocyte).

It is really not necessary to take any very definite position in regard to the various schools, because in adult life the hemopoiesis is certainly polyphyletic.

However, it is universally admitted that the polymorphonuclear elements of the leukocyte groups are formed in the bone marrow. That the metamyelocyte, myelocyte and myeloblasts are progenitors of the polymorphonuclears.

Arneth was the first to study the nucleus of the polymorphonuclear elements and devised a nuclear index (Arneth neutrophilic index). He contended that cells with the least complex nucleus are younger than the cells that have a bizarre segmentation (lobulation) of their nuclear mass. The index consists of five classes of polymorphonuclear elements (neutrophils) and in healthy adults are represented as follows:

Class I. (non-segmented forms, metamyelocytes)
Class II. (neutroprils with two lobes)
Class III. (neutrophils with three lobes)
Class IV. (neutrophils with four lobes)
Class V. (neutrophils with five lobes)

This index requires the counting of 100 neutrophils. Arneth established that in normal peripheral blood on which his index is based, the relationship of the classes is as follows:

Class I	Class II	Class III	Class IV	Class V
5%	35%	41%	17%	2%

The predominating Classes are II and III. If the index shows an increase of Class I elements it is termed "LEFT SHIFT". Should the index show an increase of Class V elements, "RIGHT SHIFT" is present.

With the index it is obviously possible to detect the presence of an abnormal number of immature and hypermature cells in the peripheral blood and in turn this will serve as an index of the leukopoietic activity of the bone marrow.

Schilling, however, contending that a shift within the Classes II, III and IV does not necessarily mean a pathologic regeneration, simplified Arneth's index by dividing the neutrophilic elements into four classes.

(1) Myeloblast, premyelocyte, myelocyte (simple round nucleus)
(2) Metamyelocyte (slight indented nucleus)
(3) Stab. or Staff form (deeply indented nucleus)
(4) Segmented neutrophils (polymorphism)

These four classes are divided up into two major groups "imma-

ture and mature". In health the immature cells including metamyelocyte and stab. forms, make up 4% of all leukocytes. The mature cells including all segmented forms, make up 63% of all leukocytes. Normal physiological total 67%. Schilling further compiled the normal physiological percentage of the remaining leukocytic elements giving a percentage relationship as follows:

B	E	M	J	St	S	L	Mo
1	3	0	0	4	63	23	6

An increase of the immature group is termed "regenerative shift" and is regarded as evidence of ill regulated leukopoiesis, though the cell entering into the peripheral circulation may be normal from the morphological point of view (metamyelocyte or juvenile shift).

While Arneth takes into account only the left and right shift, Schilling pointed out further histologic changes in the neutrophils as another important part in connection with clinical diagnosis and prognosis, the lack of segmentation of the nucleus. Schilling termed this stage "Degenerative Shift." The degenerative shift is an expression of defective function of a neutrophilic leukopoiesis as a result of noxious factors that have invaded the bone marrow and brought about histologic degeneration of the neutrophilic leukopoiesis.

Both the regenerative and degenerative shift may be observed simultaneously in the peripheral blood.

The degenerative shift is recorded in the cell scheme through the increase of pathologic "Stab" or "Staff" neutrophils. Schilling contends that the shift is the direct result of sluggish segmentation and maturation of the neutrophils for reasons stated in a preceding paragraph.

Schilling has left the interpretation of the degenerative shift in relation to the numerical increase of the Stab forms to the hemopathologist for effectively applying it to the clinical data. It has been our principle for many years to include a simple Stab shift (normal morphology of the "Stab" cells) into the regenerative shift and indicate the inhibitory segmentation process as "regenerative stab shift" pointing out to the clinician that the neutrophilic leucopoiesis is faulty and noxious factors are at work.

A "degenerative shift" is reported when disintegration of nucleus, cytoplasm and granules is observed. This is characterized by pyknosis of the nucleus, and density of the chromatin which seems to be separated in the form of large and irregular lumps, showing protrusions with or without large or small vacuoles. The cytoplasm appears to be cloudy, not sharply outlined, containing a scanty number of ill-defined basophilic granules which seem to fuse with the

cytoplasm. In a severe degenerative shift the granules stain poorly, have lost much or all oxydase ferment and form small or large granule clumps in a finely vacuolated cytoplasm. In such a stage the stab form is highly fragile and many ruptured cells are seen in a blood film.

It is obvious that the "degenerative shift" is a most valuable index of the status of the neutrophilic leukopoiesis.

Schilling proved the value of his neutrophilic index in clinical hematology beyond any doubt and today no hemopathologist would attempt to diagnose a blood picture without the index.

The old-fashioned differential count is the skeleton about which the modern hemopathologist has built the "Hemogram" that has all the ear-marks to survive any attack made by defenders of the old differential count. The Schilling "Hemogram" is defined as:

A study of the peripheral blood picture and its relationship to specific diseases, affecting the blood-forming organs.

One speaks of a Hemogram of leukemia, pernicious anemia, hemolytic icterus, infections, toxemias, etc.

What Makes Up a Hemogram?

1. The enumeration of the erythrocytes per cubic millimeter.
2. The enumeration of the leukocytes per cubic millimeter.
3. The enumeration of the reticulocytes.
4. The enumeration of the blood platelets.
5. Determination of Hb. in grams or percentage.
6. Determination of the percentage relationship of the differential cellular elements.
7. Determination of the neutrophilic (nuclear) index.
8. The qualitative study of all cellular elements (morphology, size, staining, etc.).

What Value Has a Hemogram?

1. It reveals the functional status of the hemopoietic organs. Regenerative (cell substitution) and degenerative (cell destruction); in other words normal or abnormal physiologic cell regulation.

2. It makes it possible to determine whether the hemogram is that of a specific disease of the hemopoietic organs or that of a symptomatic reaction of the organs to a specific disease.

3. The Hemogram is valuable information, diagnostic prognostic, and it is often a guide for the application of therapeutic measures.

Conclusion

A discussion of the Arneth & Schilling neutrophilic index was presented and pointed out that the Hemogram method is the more sensitive and desirable one in modern clinical hematology. The Hemogram must always be observed together with the clinical findings. The value of the Schilling Hemogram in clinical work was demonstrated by case histories.

News and Announcements

Edited by Sister Alma LeDuc,
St. Thomas Hospital, Akron, Ohio*

NATIONAL

The American Society of Clinical Laboratory Technicians was organized in Chicago, Illinois, June, 1933. It held its second annual convention in Cleveland, Ohio, June, 1934. Plans are going forward for the third annual convention to be held in Atlantic City, New Jersey, in 1935. Complete program will be published in a later issue of this journal.

1935 Tentative Program

Tentative program for Convention of A. S. C. L. T., June, 1935, to be held at Atlantic City, N. J. (Date and headquarters to be announced.)

Business meeting—Initial and final.

Report on data received from Questionnaire is to be sent to all Hospitals approved by A. C. S. and all the members of A. S. C. L. T. Questionnaire to cover all the phases of clinical laboratory procedures, as to selective tests and technic used, the difficulties encountered and any modifications successfully instituted, with reference to original article on each procedure.

Round table discussions, preceded by short story of the various topics by chairman of that section.

Topics for discussion:

Analysis of urine, feces, sputum, gastric, duodenal, and spinal fluids, transudates and exudates.

Hematology studies and technic.

Blood transfusion unit.

Serology, bacteriology and animal inoculations.

Pathology and basal metabolism.

Scientific papers and exhibits to be prominent part of the program.

Announcements

Dues for 1934-35 are $4.00 for the year, payable within 30 days after receipt of notice. This includes a year's subscription to the Publication of the Society. Secretary will issue the official certificate of Society.

Volunteers for Papers and Exhibits for Third Annual Meeting: Sr. Joan of Arc Wilson, M.T., General Chairman, Mercy Hospital, Baltimore, Md.; Miss Helen Handley, L.T., Chairman of Exhibits, Parke-Davis and Co., Detroit, Mich.; Miss Phyllis Stanley, M.T.,

* *State and local or district organizations are invited to send in any news about their meetings, elections, or other items of information. These will be printed in each issue as far as space permits.*

Chairman of Entertainment Committee, Presbyterian Hospital, Newark, N. J.

Volunteers for Publication: Correspond with Chairman of Publication.

All local and state Societies should become chartered under the National Society. The counsellors for the five districts are also acting as the Committee on Charters. They will assist you in affiliating your local societies with the American Society of Clinical Laboratory Technicians.

Counsellors—Central States: Mr. Harry Macko, L.T., Charity Hospital, Cleveland, O., Chairman. Southeastern: Madie Murphy, L.T., Hillman Hospital, Birmingham, Ala. Northeastern: Phyllis Stanley, M.T., Presbyterian Hospital, Newark, N. J. Northwestern: Helen Olson, L.T., Huron Clinic, Huron, So. Dakota. Southwestern: A. L. Coad, L.T., Coroners Office, Hall of Justice, Los Angeles, California.

The Constitution and By-Laws of the A. S. C. L. T. and excerpts of minutes of the 1934 convention at Cleveland will appear in forthcoming issues.

STATE

Alabama

The Birmingham Society of Clinical Laboratory Technicians exists but historical details have not been sent in as yet.

Illinois

The Chicago Society of Clinical Laboratory Technicians was organized April 17th, 1931. Seven registered laboratory technicians attended the first meeting which was held in Mr. George Kneeland's home. The present officers are: President, Myrtle Sand, L.T.; Vice-president, Robert Jenkins, L.T.; Secretary, Ivie Christensen, L.T., and Treasurer, Lona Jacobsen, L.T.

The Illinois Society of Clinical Laboratory Technicians was organized in Springfield, Illinois, May 17th, 1932. The present officers of the Illinois Society are: President, Ward Cade, L.T.; Vice-president, Exxa Bennett, L.T., and Secretary-Treasurer, Hester Reynolds, L.T.

New Jersey

The New Jersey Society of Clinical Laboratory Technicians was organized in May, 1933. Miss Stanley reports that last year ten meetings were held. At five meetings a pathologist or outstanding research worker gave a paper, followed by discussion. They had a social party at the Holiday season and a banquet at the end of the year. Most of the meetings were held at the New Jersey Academy of Medicine in Newark. One meeting was held in Camden, N. J.

There are 45 members with an average attendance of 25. Visitors are welcome.

Present officers are: President, Paul C. Brown, M.T.; Vice-president, Cecil H. Howen, M.T.; Secretary, Phyllis Stanley, M.T., and Treasurer, Lillian Barder, L.T.

Ohio

District No. 1 of Ohio, which comprises five counties, was organized Oct. 25th, 1933, at St. Thomas Hospital, Akron, as a unit of the American Society of Clinical Laboratory Technicians. There were then 14 registered technicians in the district and every one attended the first meeting. Student technicians are taken into the district association as associate members and visitors are welcome. Meetings are held the third Wednesday of every month except during the summer. At these meetings pathologists, staff doctors, and technicians give papers on various subjects relating to laboratory work. Present officers are: President, Sister Alma Le Duc, Ph.G., L.T.; Vice-president, Mabel Smith, L.T., and Secretary-Treasurer, Alice Finnin, L.T.

District No. 4, which comprises four counties, was organized and held its first meeting at Charity Hospital, Cleveland, Sept. 20th, 1934. Harry Macko is chairman until an election of officers is held.

The Ohio Clinical Laboratory Technicians will hold their first annual convention at the Mayflower Hotel, Akron, Ohio, Wednesday, November 21st.

Pennsylvania

There is a Western Pennsylvania Society of Clinical Laboratory Technicians of which Harry W. Langer, L.T., is Vice-president and Alma Weinheimer, L.T., is the Secretary. We have not been sent the other details or names of all the officers as yet. Miss Weinheimer reports that several meetings have been held so far.

There is also a Philadelphia Society of Clinical Laboratory Technicians but we have not received the data about them to date. Miss Frieda Ward, L.T., was the President.

Texas

The Texas Association of Clinical Laboratory Technicians was organized December 9th, 1933, in the Pan-American room of the Cunter Hotel, San Antonio, Texas. The Boxar County Association of Clinical Laboratory Technicians, of which Mr. H. A. Bardwell, L.T., R.T., is the President, started the movement for a state association, and it seems that some other counties also had local associations before the State Association was organized. The present officers of the Texas Association are: President, H. A. Bardwell, L.T., R.T.; 1st Vice-president, Pauline S. Dimmitt, Ph.G., M.T.; 2nd Vice-president, Elizabeth Pickett, L.T.; Secretary, Ida F. Levinson, B.A., L.T.; Treasurer, George T. Thomas, L.T.

To the Editor*

Milwaukee, Wis.

"We congratulate you on the progress your organization is making, and you have our very best wishes for the success of not only the organization but also of your publication."

W. A. V.

Cleveland, Ohio

"I think your idea of having a section of the journal given over to practical points relating to various standard laboratory procedures and modifications in each issue is a good one. I shall send in a note relating to rapid staining of frozen sections with steaming hematoxylin and also a note on the precautions necessary for obtaining invariable satisfactory Fontana stained preparations."

B. S. KLINE, M.D.,
Mount Sinai Hospital.

* Letters should be sent to John H. Conlin, Chairman of Publication. Such letters will be printed in forthcoming issues as far as space permits. Writers should sign their names, which will be omitted on request.